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Factors Influencing the Induction of Diapausing Egg  
Production in the Calanoid Copepod  
Diaptomus leptopus

David Piercey

A Thesis  
in  
The Department  
of  
Biology

Presented in Partial Fulfillment of the Requirements  
for the Degree of Master of Science at  
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## ABSTRACT

### Factors Influencing the Induction of Diapausing Egg Production in the Calanoid Copepod Diaptomus leptopus

David Piercey

The objective of this study was to determine the relative importance of photoperiod and temperature upon the induction of diapausing egg production in a single species of copepod found in habitats that differ in permanence.

Field studies of four populations of *Diaptomus leptopus* carried out from 1995 to 1997 reveal quite different seasonal patterns in the production of subitaneous and diapausing eggs; these distinct patterns are similar between years for three of the four ponds studied. To determine the possible roles of temperature and photoperiod on the induction of diapausing eggs, I performed a common-garden experiment in which mating pairs from each of the four populations were exposed to different temperature and photoperiod regimes: long-day (16:8h L/D) 25°C, long-day 10°C, short-day (8:16 L/D) 25°C and short-day 10°C to determine if there are differences in the onset of diapausing egg production between and within the populations in each treatment.

The populations of the occasionally temporary environments Hill and Stoneycroft exhibited a decrease in the onset of diapause under cold conditions and the overall timing of onset of diapause egg production in the experimental populations differed from that of the field populations giving indirect evidence that each population are responding to the available cues as predictors of environmental change in different ways.

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## DEDICATION

To my parents William Francis and Alma Gloria Piercey for putting me on this great earth and to Emily K. Wallace for helping me get this far, I dedicate this thesis.

Thanks.

DP

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## **Introduction**

Diapause in arthropods is defined as a dormancy in which morphological development is suppressed (whether or not growth ceases completely) through a central control, not simply by the direct action of environmental factors (Danks 1987). The ability of copepods (Class: Crustacea) to enter diapause provides an important flexibility in life-history responses to adverse biotic and abiotic conditions. Calanoid copepod populations are able to survive severe environmental conditions as the result of individual animals producing desiccation resistant diapausing eggs that can survive extended dry periods (Brewer 1964, Taylor *et al* 1990). These eggs also have the ability to remain viable after long periods of time buried in sediments (De Stasio 1989, Marcus 1990), resulting in a seed bank that can allow the copepods to recolonize the habitat when conditions become favorable once again. At present, the oldest recorded copepod egg hatched from one pond is estimated to be around 322 years old (Hairston *et al* 1995). A most interesting factor in the diapause response of calanoid copepods is that it is shown in temporary small-pond populations where water often disappears, in permanent large-lake populations, and in coastal marine populations where no water limitation is experienced. This general pattern has been investigated by many (Brewer 1964, Marcus 1982, Hairston and Van Brunt 1994), but there is no general agreement about the causative factors controlling the onset of diapause.

In this paper, I ask about the flexibility of control of onset of diapause in *Diaptomus leptopus* Forbes: will organisms enter diapause at pre-determined times perhaps as a response to photoperiod and/or temperature as seen in cyclopoids (Watson and Smallman 1971, Maier 1992), other freshwater calanoids (Hairston & Olds 1984) and marine calanoids

(Marcus 1980, 1982) or is there variability among years, perhaps as a response to other local conditions? Data reported by numerous authors often have been ambiguous and even contradictory and have implicated many factors influencing induction of diapause: e.g. proximate cues such as temperature, dilute electrolytes, photoperiod and age. The following paragraphs illustrate these various factors.

Hutchinson (1967) implicated temperature as a causative factor in differences in diapausing egg production between northern and southern populations of the calanoid copepod *Aglaodiaptomus denticornis*. He also proposed that in temporary environments, an increase in salinity or other dilute electrolytes might serve as a cue of impending catastrophic conditions.

Watson and Smallman (1971) found that two different cyclopoid populations cohabiting the water column at different times were induced to diapause at different critical photoperiods under laboratory conditions. Temperature was found to influence the critical daylength values for the transition of the response from long-day to short day for each species. The authors demonstrated that the copepods were reacting to environmental cues experienced well before the onset of the diapause in much the same manner as do insects.

Marcus (1980) raised a population of the marine calanoid *Labidocera aestiva* in the laboratory at 15°C and exposed individuals to either a long-day (16L:8D) or short-day (8L:16D) photoperiod. Animals exposed to the LD treatment produced subitaneous (immediately hatching) eggs while those exposed to the SD treatment produced diapausing eggs.

A series of experiments (Marcus 1982a) produced results that suggest that changes in photoperiod experienced by the mature female can result in the reversal from the

production of diapausing eggs back to the production of subitaneous eggs. She proposed that each individual female copepod in the population possesses a response threshold to cumulative photophases (light periods) or scotophases (dark periods). When the cumulative photophases exceed the threshold, subitaneous eggs are produced and vice-versa. She suggests the possibility that these particular copepods, *Labidocera aestiva*, produce mixed clutches during the transition phase from subitaneous to diapausing egg production.

Another series of trials (Marcus 1982b) lends support to photoperiod as the primary stimulus responsible for the type of egg produced and also shows the modifying effects that temperature does have at some photoperiods. Under short-day (8L:16D) photoperiods, subitaneous egg production was increased by an increase in temperature but females exposed to long-day photoperiods were unresponsive to changes in temperature.

Marcus (1984) sampled four populations of *L. aestiva* from different latitudes along the Atlantic coast of the U.S. ranging from Massachusetts in the north to Florida in the south. The egg production of both field-collected and laboratory-reared females was determined and compared. Southern populations rarely produced diapausing eggs regardless of the temperature and photoperiod regime experienced during rearing. Field populations also produced mostly subitaneous eggs. The northern populations tended to produce diapausing eggs under short-day photoperiods and cooler temperatures both in the laboratory and in the field.

Hairston and Olds (1984) found that for two separate populations of *Diaptomus sanguineus* found 27 km from each other but in different pond types, the females reciprocally transferred from one pond to the other continued to produce diapausing eggs in a sequence that mirrored that of the home population. The fact that these populations were

not able to adjust their egg production to reflect the pond conditions in which they were transferred into led to the conclusion that the conditions inducing diapause appear to be distinct for populations living in different pond types and that there is genetic control over induction of diapausing eggs.

Walton (1985) investigated the factors regulating the reproductive phenology of *Onchydaptomus bergei* and found that this species tended to produce diapausing eggs primarily as a function of age. When testing the effect of food levels, it was found that food shortage did not induce diapause induction but instead prevented the formation of diapausing eggs. Raising populations in various levels of dissolved oxygen did not show an inductive effect of lowered oxygen levels. He also found that there were no discernable effects of an increase in rearing density upon induction of diapausing egg production.

Hairston and Olds (1986) found three different local populations of *Diaptomus sanguineus* switching to the production of diapausing eggs at different dates. The timing of diapause appeared to correspond to the onset of seasonally harsh conditions in each pond. Controlled laboratory rearing experiments indicated that each population has a distinct range of critical daylengths at which they switched but these differ from the timing of the field populations. The results indicate that these populations utilized distinct environmental cues other than photoperiod *per se* to set the timing of onset of diapausing egg production and they propose that it is perhaps the direction and rate of change of the photoperiod that is used as the cue by the copepods much like that found in the insects.

Hairston and Olds (1987) collected samples of the calanoid copepod *Diaptomus sanguineus* from both permanent and temporary environments, collected viable eggs and reared the young in the laboratory under either “winter” (short-day, cold) conditions or

“summer” (long-day, warm) conditions. Some individuals were allowed to reproduce under the conditions they were raised under while others were transferred to the opposite regime before reproduction. Animals reared and maintained in winter conditions produced exclusively subitaneous eggs while animals reared and maintained in summer conditions produced only diapausing eggs. No female exposed to constant conditions throughout her life span ever switched from subitaneous egg production in the early clutches to diapausing egg production in later clutches. When animals were reared in winter conditions and switched to summer conditions to reproduce, they produced subitaneous eggs initially and switched to diapausing eggs in later clutches. Animals reared in summer conditions and switched to winter conditions to reproduce produced mainly diapausing eggs initially and then switched to subitaneous eggs later.

Hairston *et al* (1990) raised *D. sanguineus* in the laboratory and found that when the animals were held under controlled conditions, the population switched egg type at a different range of photoperiods than that of the field populations indicating the presence of a mediating cue other than photoperiod found only in natural conditions. Temperature was found to play a very important role in the timing of diapause onset for *D. sanguineus* with cooler temperatures resulting in more subitaneous clutches.

Ban (1992) studied diapause egg production in the calanoid copepod *Eurytemora affinis* and determined that the type of egg produced is the result of the environmental conditions experienced by the copepods during the naupliar stages. Short-day photoperiod (10L:14D) is the primary inductive cue and the effect is promoted by low temperature. It is suggested that high population density also induces diapausing egg production. Subsequent



work by Ban and Minoda (1994) showed that the accumulation of the metabolic products of *E. affinis* resulted in an induction of diapause egg production.

Calanoid copepod populations in large lakes switch to diapausing egg production later than small lake populations and temporary pond populations have the earliest switch dates (Hairston and Van Brunt 1994). Since permanent lake populations do not experience the same kinds of adverse environmental conditions as temporary populations but still exhibit the diapause trait, perhaps the expression of diapause has not been selected against in bodies of water where it is not adaptive.

In an excellent study upon the interaction of photoperiod and temperature in the timing of diapause egg production (Hairston and Kearns 1995), the importance of temperature as an inductive cue was demonstrated in the calanoid copepod *D. sanguineus*. Temperature was shown to be an important modifier of the critical daylength at which the copepods were induced to produce diapausing eggs. The pattern of diapausing egg production by the natural population was able to be accurately reproduced in a laboratory-reared population.

Studies on cladocerans have produced some interesting results about important factors influencing the production of ephippial (diapausing) eggs. While studying the cladoceran *Daphnia*, Stross (1969) found that the primary inductive factor is daylength and this was mediated by temperature and rearing density. At longer daylengths, the effects of temperature are less important than the density of animals in the culture. Hobæk and Larson (1991) showed that induction of diapause is a complicated 2-step process that involves the induction of males first and then mictic females induced. Kleiven *et al* (1992) when studying sexual reproduction in *Daphnia magna*, found that induction of sexually-produced

eggs occurred only when three stimuli (short daylength, crowding and food shortage) were present but no ephippia (resting eggs) were ever produced by the presence of only two of these three factors. The results indicate that the animals were able to sense many different environmental parameters simultaneously and respond only when the overall cumulative changes exceeded a threshold value.

Tauber *et al* (1986) state that for insects, the expression of diapause has much intraspecific variability. It can be due to the genetic makeup of the species or it can be related to variability in the environmental conditions experienced prior to or during diapause. The diapause-inducing stimuli are only perceived during specific, genetically determined stages. In some species, the stages can be separated within the same generation or between generations whereas other species can have an overlap between the stages. For calanoid copepods, the sensitive stage of induction can be no longer than the interclutch interval (time between the production of eggs in the ovary of the female) since the females can produce different egg types from one clutch to the next (Hairston and Munns, 1984). For *Diaptomus leptopus*, the interclutch interval at 18°C is  $0.9 \pm 0.2$ d (Watras and Haney, 1980).

In general, the role of the environment in the induction of the diapause response of arthropods may be much more than just a simple temperature-photoperiod reaction. It is hypothesized by Zaslavski (1996) that most invertebrates have a 'managing' mechanism that integrates the photoperiodic information gathered by the photoperiodic clock with other sensory inputs in order to determine the type of response to a given set of conditions. Several studies upon various species of aquatic zooplankton lend support to this view: e.g. upon different species within one pond (Watson and Smallman 1971, Carter 1974), within a single species in different ponds (Hairston and Olds, 1984) and within a single species that

has a latitudinal separation between populations (Marcus, 1984). This would indicate that the populations are reacting to conditions that are distinct for each environment in different ways.

While it is clear that there are many factors that are involved in the induction of diapause egg production in freshwater copepods, temperature and photoperiod appear to be the dominant contributors to the overall cueing of the diapause response. The environment that the copepods reside in certainly provides other factors that can enhance or suppress the onset of diapause and it is the importance of these factors over and above that of photoperiod and temperature that this research will attempt to address.

### Objectives

Using four populations from a gradient of habitats ranging from a temporary environment that dries completely in summer to a small, deep permanent lake, a 3-year field study and a 4-month laboratory experiment were performed to test the hypothesis that each population is reacting to specific local conditions provided by their home pond and that the temperature and photoperiod should assume secondary importance.

The main objectives of this study are to answer the following questions:

1. Are the patterns of diapausing egg production found in the four natural populations of *D. leptopus* the result of selection to respond to the differing degrees of permanence of the environments?
2. Can any differences in the patterns of diapausing egg production between populations be related to one or more of the physical/chemical parameters found in these ponds?

3. Will temperature and photoperiod influence these different populations in the same way under the controlled conditions of a laboratory common-garden experiment and if so, will any differences in diapause induction disappear when the populations are maintained under identical conditions?

## **Materials & Methods**

### Study Organism

*Diaptomus leptopus* S.A. Forbes 1882 is a North American freshwater calanoid copepod species that can be found at all altitudes in both temporary and permanent environments from coast to coast across Canada and the northern U.S. as far south as Virginia on the east coast, Oregon on the west coast, throughout the Rocky Mountains and eastern Alaska (Ward & Whipple, 1959). It is obligately sexual and must mate before each clutch is produced (Watras and Haney, 1980). It is known to have a variable female-biased sexual size dimorphism among populations in the Montreal region (DeFrenza *et al.* 1986).

### Study Sites

Populations of *Diaptomus leptopus* were studied from four different locations differing in pond permanence.

- a) **Quarry pond** (45°25'45"N, 73°52'30" W) is a former quarry that ceased operation in the early 1900's and is located within the Morgan Arboretum, Ste-Anne-De Bellevue, Qc. The main source of freshwater is the spring runoff and as a result, the pond normally dries up completely in mid-summer. It has a maximum depth of approximately 1.5m. Subsequent fillings due to rainfall do not result in an appearance of a measurable population of *Diaptomus leptopus*. This is a seasonally temporary environment.

- b) **Stoneycroft** ( 45°25'44"N, 73°56'22"W) is a shallow natural pond found within a bird sanctuary adjacent to the Morgan Arboretum. It has a maximum depth of 0.6m and does not normally dry up during the season but likely freezes to the bottom in winter. I will refer to it as an occasionally temporary environment.
- c) **Hill Pond** (45°25'50"N, 73°56'45" W) is a man-made fire-control pond located within the Morgan Arboretum that was created around 1956. It has a maximum depth of 2.4m and occasionally freezes to the bottom in winter (E.J. Maly, *pers. comm.*). I will refer to it as an occasionally temporary environment.
- d) **Lac Geai** ( 45°59'41"N, 73°59'34"W) is a permanent lake found near the University of Montreal's field station 17 km northeast of St-Hippolyte, Qc. The maximum depth is ~5.5m. This lake has no history of drying up completely and has an overwintering population of *D. leptopus*. I will refer to this as a permanent environment.

### Zooplankton Collection

The zooplankton used in this study was collected either weekly or every second week using a Wisconsin-type tow net (80 µm mesh), thrown and towed horizontally just below the water surface. Samples were transported in 20 liter plastic carboys and sorted upon return to the lab or stored in a 10°C coldroom for a maximum of 48 hours before sorting. Two types of preserved samples were collected on each date for each pond; qualitative samples collected from horizontal tows using a Wisconsin-type plankton net, preserved with a 10% Formalin mixture and quantitative samples collected using a 4-liter flexible drop-tube sampler (Knoechel and Campbell, 1992), preserved by the addition of 95% ethanol.

### Plankton Sorting

In late spring, animals were collected from each of the four study ponds and were continuously isolated in sexually-segregated pure stock cultures as they moulted from the copepodid V stage to sexually mature adults. To isolate the plankton samples into pure cultures, 400 - 500 ml of water was removed from the storage carboy and poured into a flat-bottom white polycarbonate sorting tray to aid in visual identification. Individual copepods were removed from the sorting tray using 2.5ml glass transfer pipettes and were placed into individual wells of a 9-spot pyrex depression plate. Animals were sexed with the aid of a Zeiss binocular dissecting microscope and stored in 4-liter glass jars filled with filtered pond water.

As a result of the earlier development and emergence of male *Diaptomus leptopus* from all the study populations (Nishikawa & Maly 1996), we were able to remove most of the males from the early season samples and isolate them in pure cultures before the females became sexually mature. The sample jars containing females were sorted several times a week in order to prevent the sexually mature females and newly emerging females from being mated by undetected, slow-developing males. The isolated male and female *D. leptopus* were stored in a 10°C coldroom and fed a dilute solution of YCT (Yeast, Cerophyll, Trout chow) medium (Environment Canada, 1990) twice a week until required for experimental work.

### Reproductive Phenology

Egg-bearing (clutched) females were isolated from the weekly field-collected samples within 24 hours of collection and placed individually in 6ml wells of plastic tissue

culture plates containing 37  $\mu\text{m}$  mesh-filtered pond water. Usually 48 females from each date were used but on occasion, smaller samples were used when animals were scarce. Utilizing the method of Hairston and Olds (1984), the clutched females were observed for a 14-day period. Normally, the subitaneous clutches began hatching while still attached to the female within the first five days of isolation while the clutches composed of diapausing eggs were dropped by the female either as one cohesive clutch or freely scattered on the bottom of the well. Eggs that remained unhatched after 14 days were considered to be diapausing eggs and were collected and stored in a 96-well tissue culture plate and checked periodically for late hatching.

#### Pond Physical Conditions

The physical conditions of the water of each pond were determined with the aid of a Corning Checkmate® M90 handheld metering system with detachable probe elements. The parameters measured were: 1) temperature ( $^{\circ}\text{C}$ ), 2) conductivity ( $\mu\text{S}$ ) and 3) total dissolved solids ( $\text{mg/l}$ ). Measurements were taken weekly or bi-weekly from May to September at each sample site of the study between 1995 and 1997.

#### Diapause Induction

Four sets of thirty-six mating pairs from each of the four ponds were isolated individually into 15  $\times$  85mm glass culture tubes containing 8 ml of water. Each set of tubes were randomly placed into test-tube racks comprising a 12  $\times$  12 array within a light-excluding cardboard enclosure. Illumination was provided by a 40-watt incandescent lamp controlled by a programmable timer set for either a long-day (16L:8D) or short-day

(8L:16D) photoperiod. Cold temperature treatments were placed in an 8°C walk-in coldroom while the warm temperature treatments were placed on benches in the laboratory with an average ambient temperature of  $23 \pm 2^\circ\text{C}$ .

Tubes were checked once a week to determine the status of each mating pair. All females found to be carrying a clutch were recorded as clutched females, the presence of nauplii was recorded as a hatching and any eggs found at the bottom of the tube were collected and stored in 96-well tissue-culture plates and observed frequently for hatching within the two weeks after extrusion. Egg not hatched after two weeks were recorded as diapausing eggs but were checked occasionally for late hatching.

The tubes were cleaned of debris and waste weekly and were fed twice-weekly with 1ml of YCT medium diluted 4:1 with deionized water to give a final food concentration per tube of approximately 36 mg solids/liter. In the case of dead animals, males were replaced immediately from either the initial stock cultures collected in late spring or from samples collected later in the season. Dead females however, were usually replaced only from the initial stock cultures except for the copepods from Lac Geai, in which copepodids (juvenile stages) were isolated from the early summer samples and raised to adulthood. When no animals were available for replacement, the tubes requiring animals were removed from the experiment.

### Statistical Analysis

Data transformations, manipulations, G-test and Chi-square calculations were done with the aid of Microsoft Excel v.4.0a and Excel v.7.0. All ANOVA and regression analysis was done with the aid of SPSS Professional Version 7.0.



## Results

### Reproductive Phenology

The timing of the onset (the date on which 50% of the sampled population begin production of diapausing eggs) is related to the permanence of the environment occupied by each population (Table 1). The results of logistic regression analysis indicate that there are significant differences in the onset of diapause egg production among the three temporary ponds ( $\chi^2 = 429.279$ ,  $p_{(2)} = 0.000$ ).

In three years of study, the samples from the temporary pond population of Quarry (Fig. 1a) exhibited a sharp increase in proportion of diapause clutches produced within the week after the summer solstice, the longest day of the year, but no differences could be detected for the day of onset ( $\chi^2=0.098$ ,  $p_{(1)}= 0.744$ ) among the three years. Stonecroft (Fig. 1b) has a significant difference in the timing of induction between years ( $\chi^2=38.64$ ,  $p_{(2)}= 0.000$ ) but the timing of onset in 1995 ( $\chi^2=0.481$ ,  $p_{(1)}= 0.488$ ) and 1996 ( $\chi^2=0.835$ ,  $p_{(1)}=0.3608$ ) were not responsible for the difference. However, the day of onset in 1995 and 1996 differed significantly ( $\chi^2=64.567$ ,  $p_{(1)}= 0.000$ ). The logistic regression indicates that Hill pond (Fig. 2a) exhibits a consistent timing of onset as indicated by a perfect fit of the logistic regression (Nalgerke  $R^2 = 1.000$ ) for the years 1995-1997. Unpublished data from a 1989 study indicates that the onset of diapause egg production in Hill pond has remained consistent over time. The samples from the population of the permanent environment Lac Geai (Fig.2b) indicate that little to no diapause egg production occurs between May and September, the period during which all sampling was carried out.

Table 1 : Diapause Onset Date – Natural Population Field Data. The induction date is the date on which 50% of the sampled population produces diapausing eggs. The % diapause indicates the percentage of females observed that produced diapausing eggs. The temperature is average water temperature of the pond on the induction date. The letters y and z in the table indicates: y) diapause levels detected never exceeded 20% (See methods), and z) no diapause detected during sampling season. Stoneycroft 1997 data incomplete.

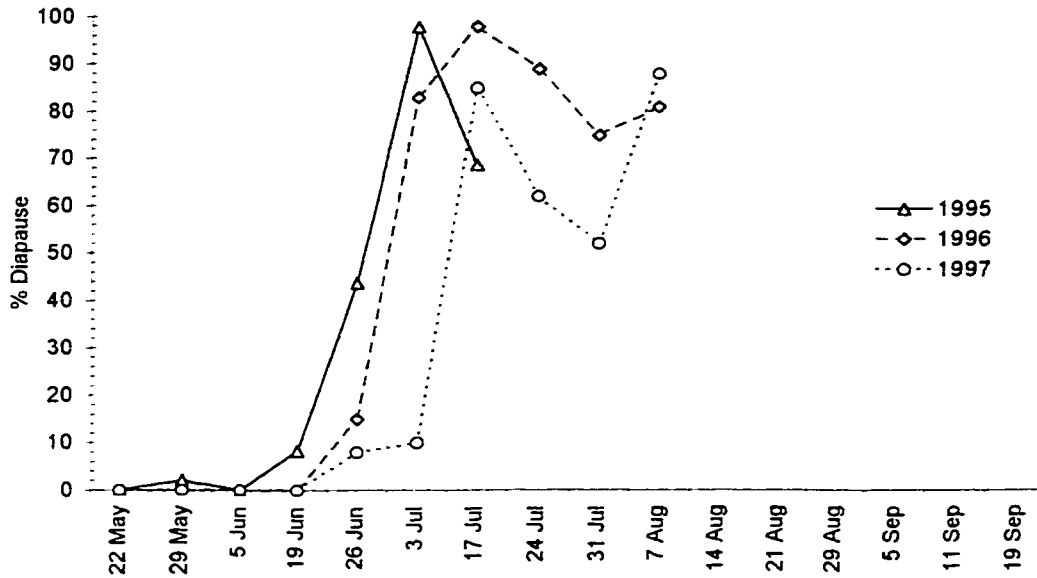
Pond	Year	Induction Date	% Diapause	Temp.(°C)	Day Length (h)
Quarry	'95	June 26	98	23.1	15.71
	'96	June 26	83	18.2	15.70
	'97	June 30	85	23.5	15.67
Stoneycroft	'95	August 14	79	24.4	14.22
	'96	July 31	54	21.0	14.80
Hill	'95	August 14	95	21.2	14.22
	'96	August 14	83	20.9	14.18
	'97	August 11	69	24.3	14.33
Lac Geai	'95	y	y	y	y
	'96	y	y	y	y
	'97	z	z	z	z

Figure 1 : Pond diapause data 1995-1997. a) Quarry pond, Morgan Arboretum,  
b) Stoneycroft, Morgan Arboretum. The % diapause represents the proportion of  
clutches that do not hatch after a two-week incubation at room temperature.  
Sample sizes are between  $n = 12$  and  $n = 48$ .

Figure 1. Pond Diapause Data 1995-1997.

a) Seasonally Temporary Environment

Quarry, Morgan Arboretum, Ste.-Anne-De-Bellevue, Qc. 45°25'45"N, 73°52'30"W.



b) Occasionally Temporary Environment

Stoneycroft, Morgan Arboretum, Ste.-Anne-De-Bellevue, Qc. 45°25'44"N, 73°56'22"W.

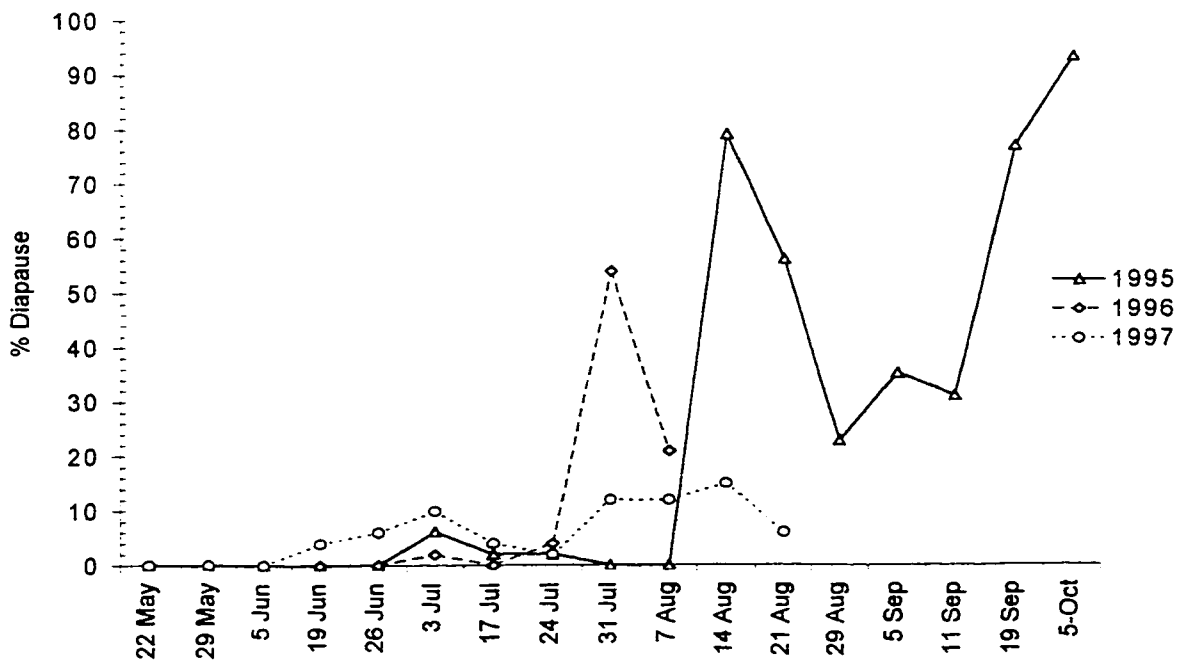
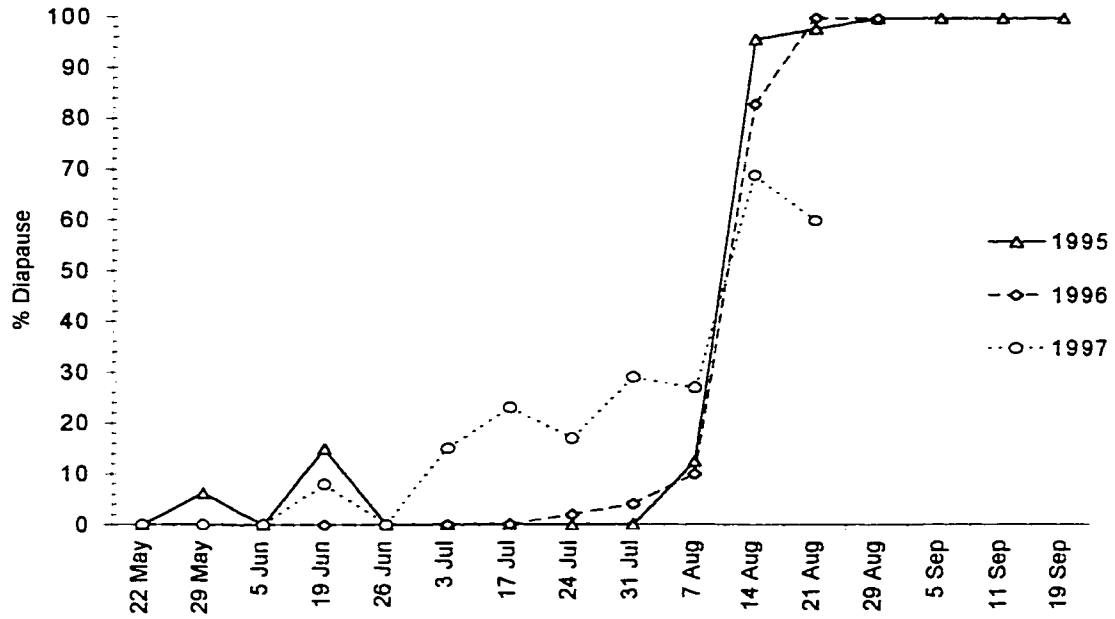


Figure 2 : Pond diapause data 1995-1997. a) Hill pond, Morgan Arboretum, b) Lac Geai, St. Hippolyte. The % diapause represents the proportion of clutches that do not hatch after a two-week incubation at room temperature. Sample sizes are between  $n = 12$  and  $n = 48$ .

Figure 2. Pond Diapause Data 1995-1997.

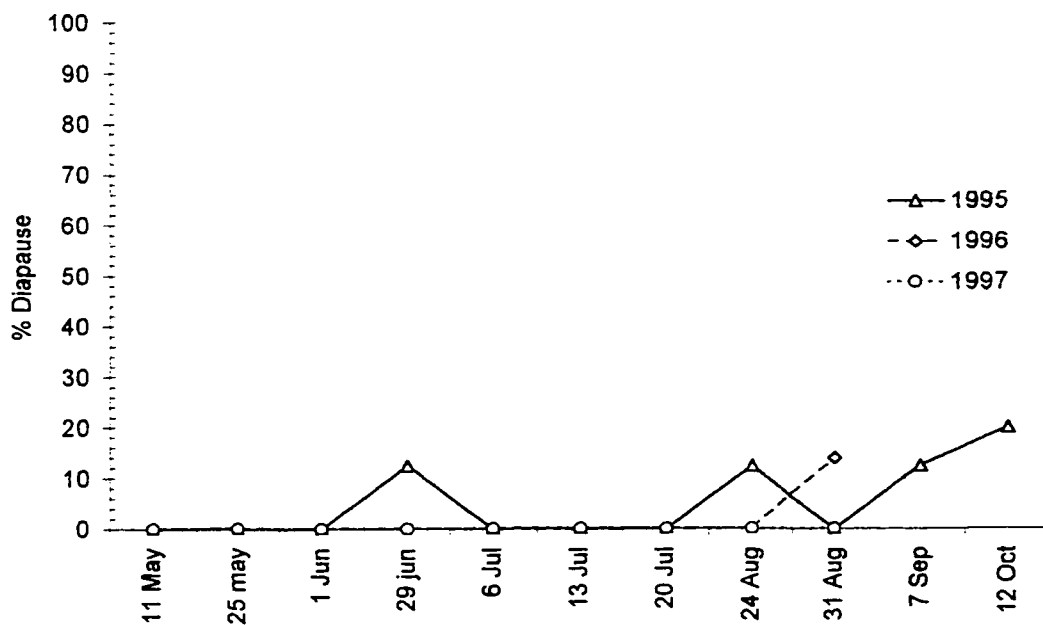
a) Occasionally Temporary Environment

Hill Pond, Morgan Arboretum, Ste-Anne-De-Bellevue, Qc. 45°25'50"N, 73°56'45"W.



b) Permanent Environment

Lac Geai, St-Hippolyte, Qc. 45°59'41"N, 73°59'34"W.



### Pond Physical Conditions (Figs. 3-6)

Significant differences between years in the timing of diapause onset were found only in Stoneycroft (Fig. 4) and these physical condition data were utilized for further analysis. Tests for between-year differences were performed using simple factorial ANOVA and Tukey post-hoc tests were used to determine the years that significantly differed.

There were no significant differences found between years for temperature ( $F=0.773$ ,  $p_{(2)}= 0.472$ ) but significant between-year differences were found for both conductivity ( $F = 6.987$ ,  $p_{(2)}= 0.003$ ) and total dissolved solids ( $F = 3.792$ ,  $p_{(2)}= 0.031$ ). Tukey tests indicate that the 1996 means of both conductivity and total dissolved solids were significantly lower than the 1995 and 1997 means. There were no significant differences between the latter two means.

### Timing of Diapause Onset

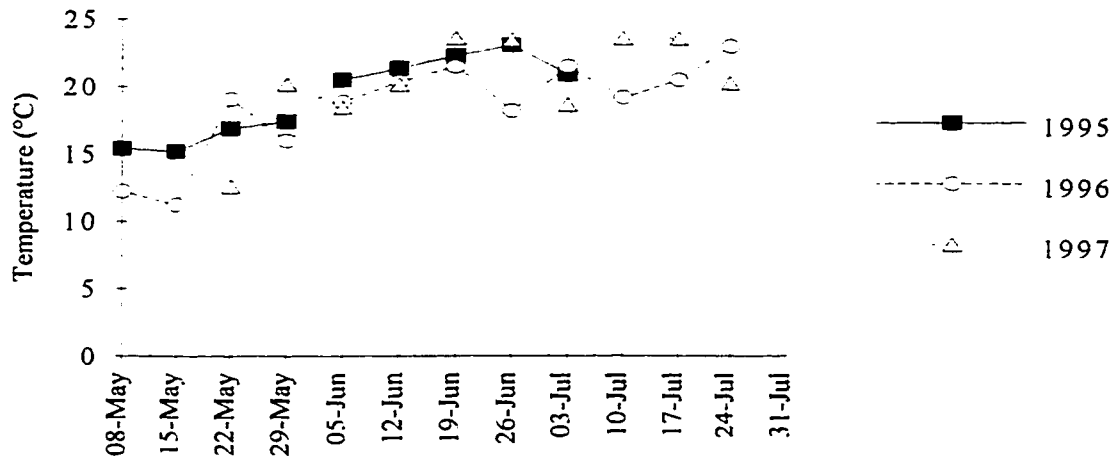
The timing of diapausing egg production under controlled conditions differed greatly from the timing of the natural populations (Table 2). The experimental population from Quarry began producing diapausing clutches 2-3 weeks later than the natural population, while the Hill Pond and Stoneycroft experimental populations began production of diapausing clutches from 1-6 weeks earlier than the natural populations depending on the treatment. Although several individual females from Lac Geai did produce diapausing clutches, the weekly proportion of diapausing to total clutches never exceeded 50%.

Figure 3 : Physical pond conditions 1995-1997 : Quarry, Morgan Arboretum.  
a) Temperature (°C), b) Conductivity (μS), c) Total Dissolved Solids (mg/l).

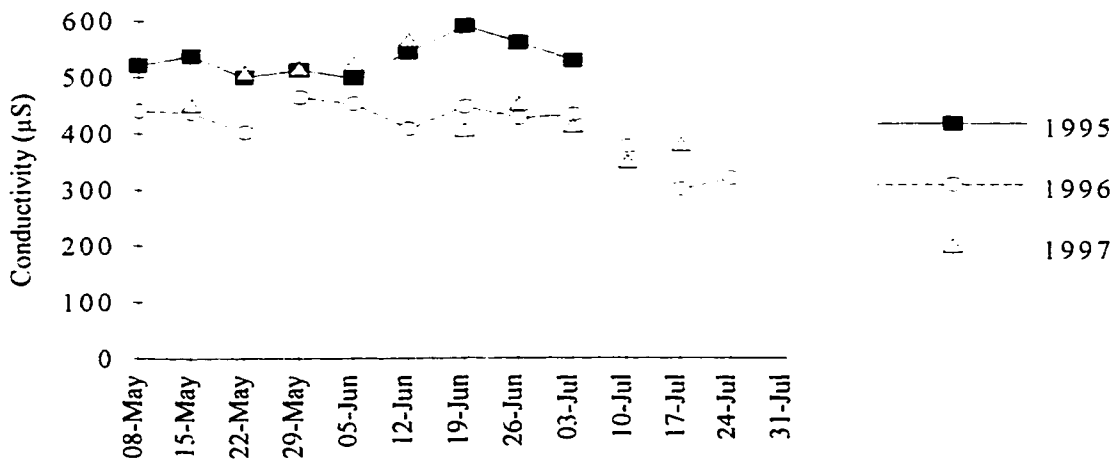


Figure 3 : Physical Pond Conditions 1995-97 - Quarry

a) Temperature



b) Conductivity



c) Total Dissolved Solids

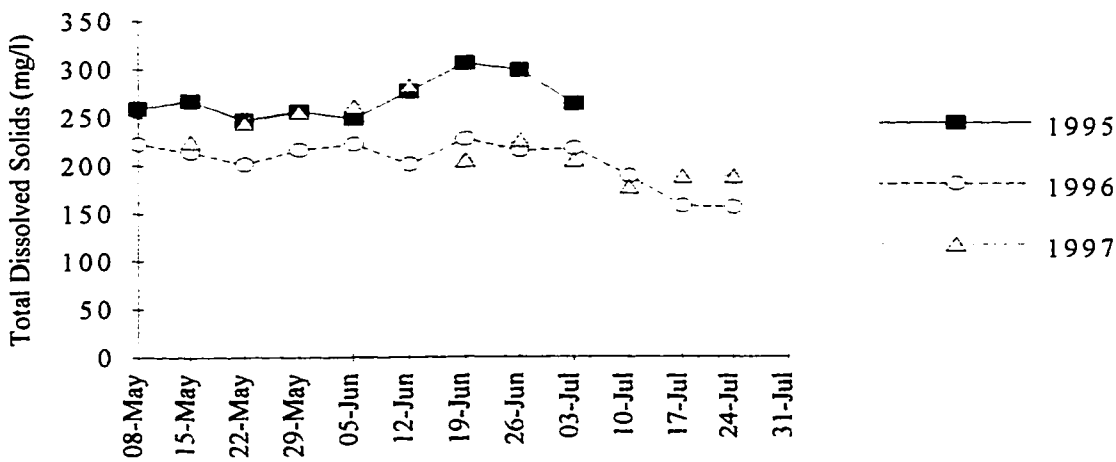
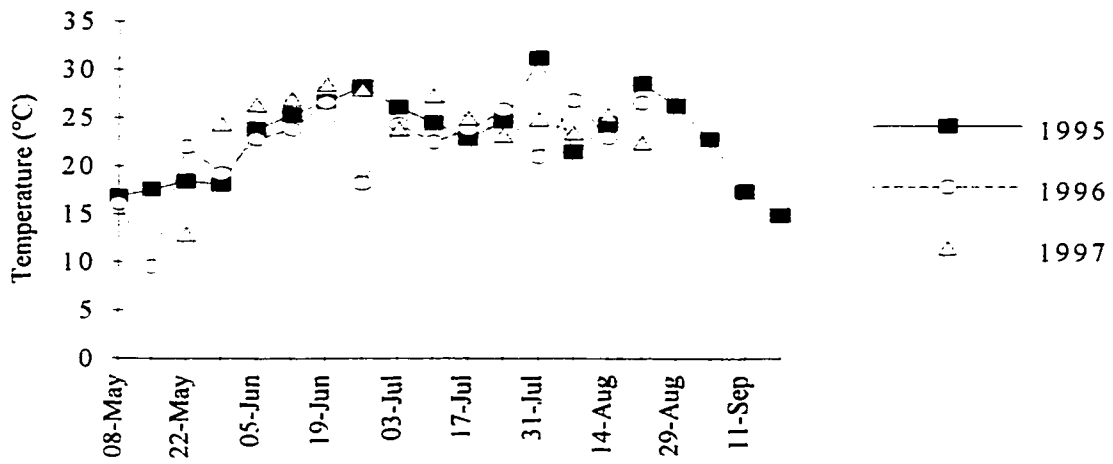


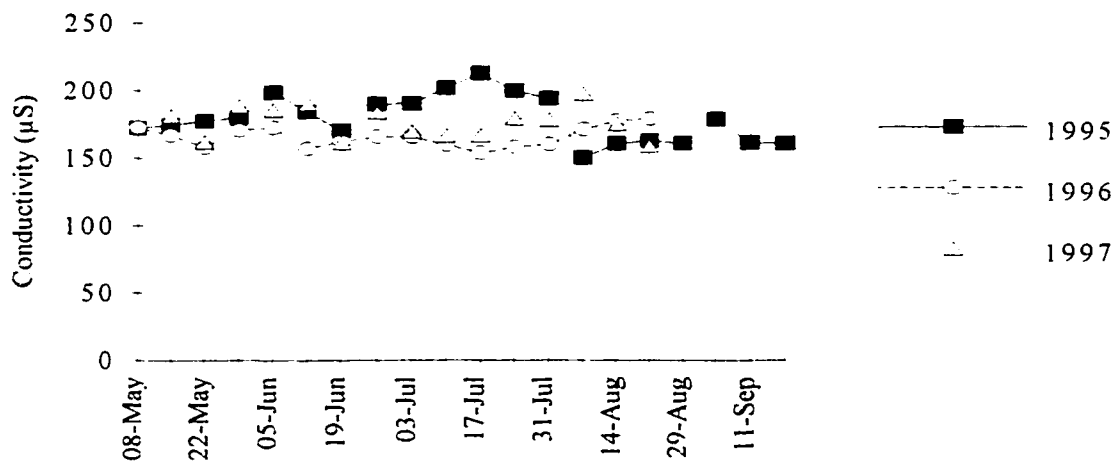
Figure 4 : Physical pond conditions 1995-1997 : Stoneycroft, Morgan Arboretum.  
a) Temperature ( $^{\circ}\text{C}$ ), b) Conductivity ( $\mu\text{S}$ ), c) Total Dissolved Solids ( $\text{mg/l}$ ).

Figure 4 : Physical Pond Conditions 1995-97 - Stoneycroft

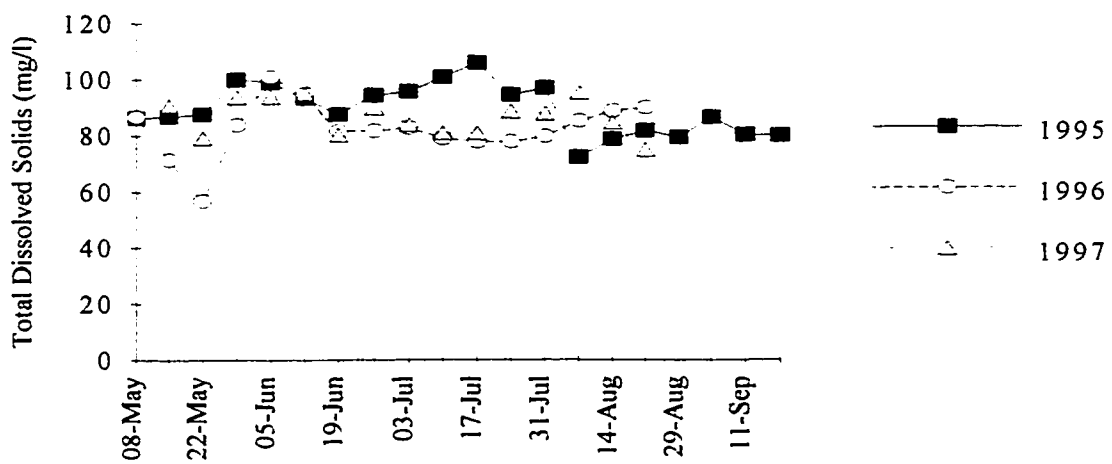
a) Temperature



b) Conductivity



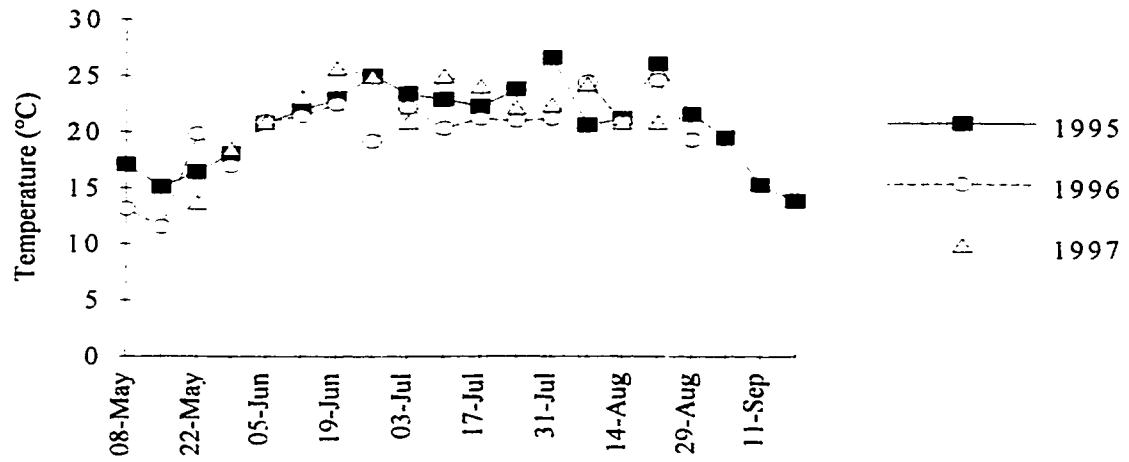
c) Total Dissolved Solids



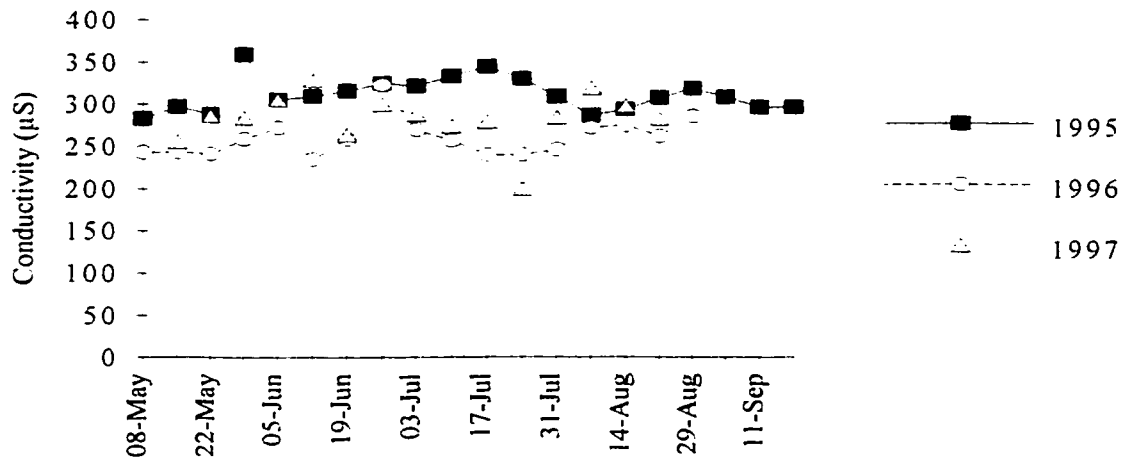
**Figure 5 : Physical pond conditions 1995-1997 : Hill Pond, Morgan Arboretum.**  
a) Temperature (°C), b) Conductivity (μS), c) Total Dissolved Solids (mg/l)

Figure 5 : Physical Pond Conditions 1995-97 - Hill Pond

a) Temperature



b) Conductivity



c) Total Dissolved Solids

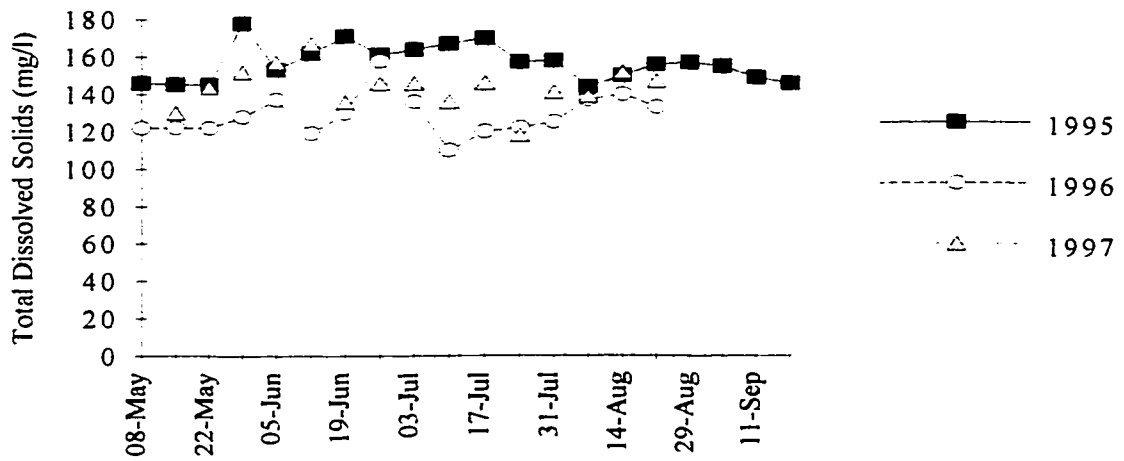
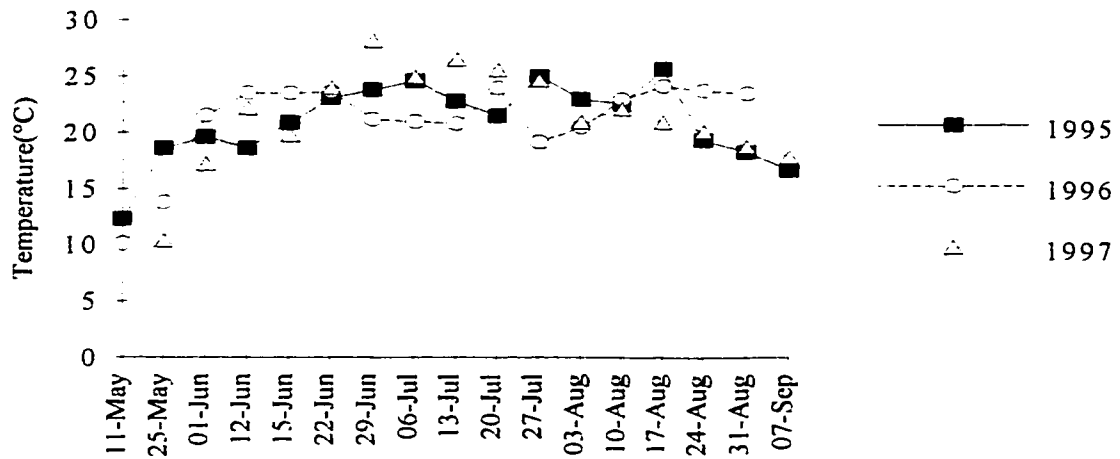


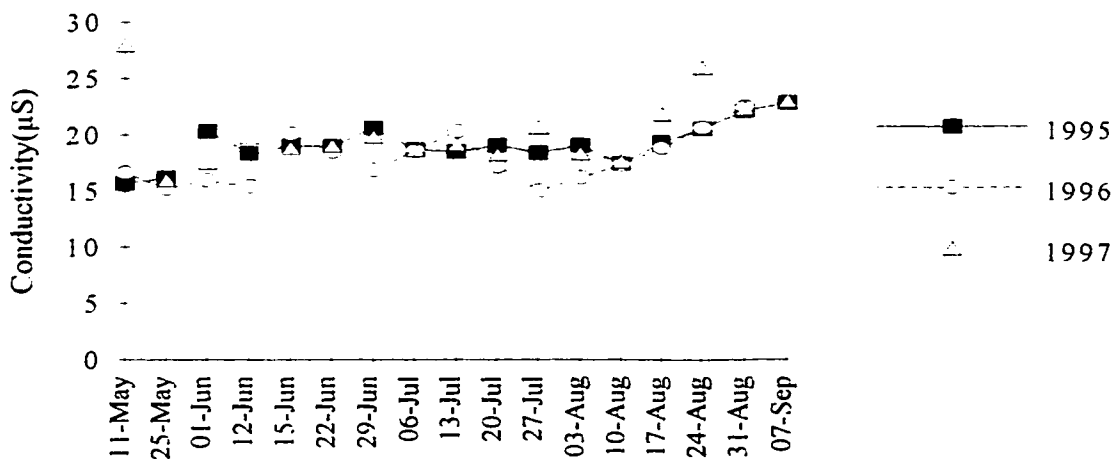
Figure 6 : Physical pond conditions 1995-1997 : Lac Geai. a) Temperature ( $^{\circ}\text{C}$ ),  
b) Conductivity ( $\mu\text{S}$ ), c) Total Dissolved Solids ( $\text{mg/l}$ ).

Figure 6: Physical Pond Conditions 1995-97 - Lac Geai

a) Temperature



b) Conductivity



c) Total Dissolved Solids

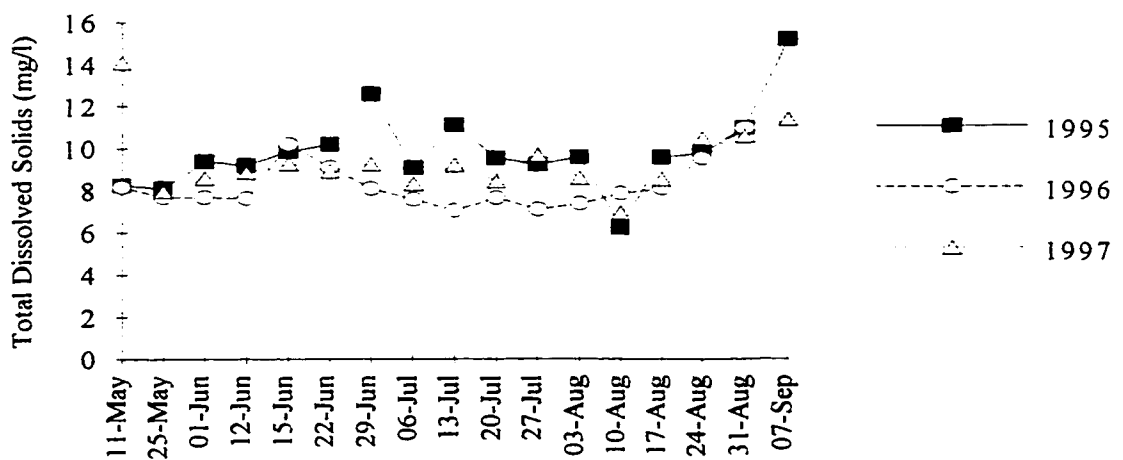


Table 2 : Diapause Onset Date - Induction Experiments 1996. See text for details on treatments. Experiment onset date is the sampling date in which 50% or more of the egg clutches collected were determined to be diapausing (See methods). Field onset date is the sampling week on which the percentage of diapausing clutches collected from the natural populations were equal to or greater than 50%. Difference is the experiment onset date minus the field onset date ( $\pm 3d$ ) in weeks.

Pond	Treatment	Experiment Onset Date	Field Onset Date	Difference (weeks)
Quarry	SD 10	July 15	June 24	+ 3
	LD 10	July 8		+ 2
	SD Room	July 11		+ 2
	LD Room	July 11		+ 2
Stoneycroft	SD 10	July 22	July 31	- 1
	LD 10	July 2		- 4
	SD Room	July 11		- 3
	LD Room	July 11		- 3
Hill	SD 10	July 22	August 14	- 3
	LD 10	July 8		- 6
	SD Room	July 24		- 3
	LD Room	July 11		- 5
Lac Geai	SD 10	none	none	none
	LD 10	none		none
	SD Room	none		none
	LD Room	none		none



### Diapausing Egg Production

As in the pond populations, the overall pattern of diapause egg production of animals maintained in controlled common-garden conditions remains similar (Table 3) with the three non-permanent populations producing large numbers of diapausing clutches while the permanent population produced mostly subitaneous clutches.

A 4x4x2  $\chi^2$  contingency test was used to test for mutual independence of egg type, pond and treatment. The results of the test indicate that the three variables lack independence ( $\chi^2 = 44.03$ ,  $p_{(3)} < 0.005$ ). Partial tests of independence show that egg type is not independent of pond and treatment ( $\chi^2 = 205.58$ ,  $p_{(1)} < 0.001$ ), the ponds are not independent of egg type and treatment ( $\chi^2 = 193.38$ ,  $p_{(3)} < 0.001$ ), and the treatments are not independent of pond and egg type ( $\chi^2 = 217.81$ ,  $p_{(3)} < 0.001$ ).

To determine which of the ponds and treatments differ within the analysis, the chi-squares were decomposed further to a series of 2x4 tests. Of the four ponds tested, neither the permanent Lac Geai ( $\chi^2 = 4.72$ ,  $0.25 < p_{(3)} < 0.10$ ) nor the seasonally temporary Quarry ( $\chi^2 = 3.01$ ,  $0.50 < p_{(3)} < 0.25$ ) environments showed any effect of treatment. The occasionally temporary environments Hill pond ( $\chi^2 = 12.09$ ,  $p_{(3)} < 0.005$ ) and Stoneycroft ( $\chi^2 = 217.81$ ,  $p_{(3)} < 0.001$ ) however, do show a significant effect of treatment. For Hill pond, the test of three treatments combined (SD10, SD Room and LD Room) is not significant ( $\chi^2 = 0.57$ ,  $p_{(2)} > 0.05$ ), but when the three combined treatments were tested against the LD10 treatment, it results in a significant treatment effect ( $\chi^2 = 9.35$ ,  $p_{(1)} < 0.005$ ). Similarly for Stoneycroft, the test of the combined SD10, SD Room and LD Room treatments was not significant ( $\chi^2 = 0.016$ ,  $p_{(2)} > 0.05$ ) but when tested against the LD10 treatment, a significant treatment effect is found ( $\chi^2 = 11.17$ ,  $p_{(1)} < 0.001$ ).

Table 3 : Overall Production of Diapausing Eggs. See text for details on treatments. Reproductive females denotes the number of females per pond per treatment that produced clutches.

Pond	Treatment	Reproductive Females (n =)	Diapausing Clutches	Total Clutches	Diapausing Clutches/Female
Quarry	SD 10	31	76	96	2.452
	LD 10	33	62	78	1.879
	SD Room	34	53	75	1.559
	LD Room	47	81	92	1.723
Stoneycroft	SD 10	29	59	79	2.034
	LD 10	37	35	67	0.946
	SD Room	49	88	143	1.796
	LD Room	50	72	101	1.440
Hill	SD 10	39	78	100	2.000
	LD 10	34	24	52	0.706
	SD Room	44	67	88	1.523
	LD Room	52	70	98	1.346
Lac Geai	SD 10	34	15	106	0.441
	LD 10	34	3	65	0.088
	SD Room	19	7	25	0.368
	LD Room	34	5	40	0.147

To summarize, Hill and Stoneycroft show a significant effect of treatment upon type of egg produced (diapause or subitaneous) and in these ponds the lowest output of diapausing clutches occurs in the LD10 treatment.

#### Reproductive females producing only subitaneous eggs

Samples of the natural populations from Quarry (Fig. 1a) indicate that some proportion of the population continues to produce subitaneous eggs after the general population has switched over to the production of diapausing eggs. In the common-garden induction experiment, this pattern remained but only the populations from Stoneycroft and Hill showed any differences between treatments. The results of the Chi-square contingency test indicate that when these two populations are maintained under long-day cold conditions, more individuals continue to produce exclusively subitaneous clutches than under the other three treatments (Table 4). Although this treatment has no ecological relevance, the results indicate that lower temperature can delay the onset of diapause egg production.

When examining population differences among treatments, significantly more individuals from the Lac Geai population produced only subitaneous eggs than any of the other populations regardless of the treatment (Table 5). The temporary populations differed only under the long-day cold treatment in which Quarry had significantly fewer individuals producing only subitaneous eggs than did Stoneycroft and Hill.

Table 4 : Differences within populations of females producing only subitaneous eggs  
 LD10 =16L:8D photoperiod at 10°C. Others = SD10, SD Room and LD Room.  
 ftr – fail to reject. ns – not significant.

Pond	$\chi^2$ Calc.	$\chi^2_{df=3}$	$H_0: p_1 = p_2 = p_3 = p_4$	Differences
Quarry	2.820	7.815	ftr $H_0 : 0.50 < P < 0.25$	ns
Stoneycroft	12.246	7.815	Reject $H_0 : P < 0.01$	LD 10 > others
Hill	11.893	7.815	Reject $H_0 : P < 0.01$	LD 10 > others
Lac Geai	6.616	7.815	ftr $H_0 : 0.10 < P < 0.05$	ns

Table 5 : Differences within treatments of females producing only subitaneous eggs.  
 See text for details on treatments. G = Lac Geai, Q = Quarry, S = Stoneycroft,  
 H = Hill.

Treatment	$\chi^2$ Calc.	$\chi^2_{df=3}$	$H_0: p_1 = p_2 = p_3 = p_4$	Differences
SD 10	44.30	7.815	Reject $H_0$ : $P < 0.001$	$G > Q = S = H$
LD 10	46.754	7.815	Reject $H_0$ : $P < 0.001$	$G > S = H > Q$
SD Room	22.215	7.815	Reject $H_0$ : $P < 0.001$	$G > Q = S = H$
LD Room	70.606	7.815	Reject $H_0$ : $P < 0.001$	$G > Q = S = H$

### Reverse Switching of Clutch Type

In all four populations and generally within all four treatments, a large proportion of individuals switched back to the production of subitaneous eggs after having already produced diapausing clutches (Table 6). The occurrence of reverse switching is found to be independent of the pond ( $G = 0.705$ ,  $p < 0.05$ ).

### Minipause

While subitaneous eggs typically hatch within two weeks of extrusion and “normal” diapausing eggs need a long refractory period and a cold shock in order to complete embryogenesis, the *D. leptopus* population of Lac Geai appears to produce a slow hatching egg that requires a refractory period of seven to eleven weeks under lab conditions before hatching will occur. Unfortunately, all clutches that were classified as diapausing were collected and stored together and so the number of females producing the delayed-hatching clutches is not known. This delay in hatching is unique to this population and has not yet been found in any of the other local populations.

Table 6: Reverse Switching of Clutch Type. Reverse switching is the return to the production of subitaneous (hatching) eggs after having previously produced diapausing eggs. Switching is independent of pond of origin ( $G = 0.705$ ,  $p < 0.05$ ).

Pond	# females w/ multiple clutches	# reverse switches	% switch
Quarry	102	25	24.5
Stoneycroft	97	31	32
Hill	90	25	27.8
Lac Geai	51	15	23.5

## **Discussion**

### Temporary vs. Permanent Habitats

The timing of onset of diapausing egg production remained consistent for three of the four populations over the three years of collection. Quarry pond is the one clearly temporary environment of the four study sites, as it dries up completely every summer before the end of July. Over three seasons of data collection, the Quarry population switched to the production of diapausing eggs at essentially the same daylength ( $15.69\text{h} \pm 1.2 \text{ min.}$ ) regardless of the pond temperature. This daylength occurs after the summer solstice, June 21, and one cannot reject the hypothesis that the population is utilizing the decrease in daylength as a cue to begin producing diapausing eggs. The pond has been relatively undisturbed since the quarry ceased operations in the late 1800's and any population that has utilized this habitat would have faced the same catastrophic end of the pond at some point of the summer for every generation of their existence at this location.

The population of Hill Pond also had a very consistent timing of diapause egg onset ( $14\text{h } 15.3\text{m} \pm 4.5\text{m}$ ) over the three years of this study and data from a 1989 study also showed the same timing of onset. This site is beside a road and is subject to frequent disturbance by swimming dogs but never dries up completely and does not always freeze to the bottom in winter. Since there is no constraint imposed by an imminent catastrophe such as pond drying and the environment does persist long after the copepods have switched to the production of diapausing eggs, it is likely that this population must be reacting to a different cue in the local environment other than just a decreasing photoperiod to induce the switch to diapausing egg production.



Stoneycroft is a pond located in a shallow basin. It has never dried up in recent years but it does freeze completely to the bottom in winter due to its shallow depth. The population of this site have shown the largest variability in timing of onset with a difference in daylength of 17.4 minutes between the two sample seasons of 1995-1996. The 1997 population had not yet switched to diapausing egg production by mid-August when collections ended so a definitive timing of a switch is not available. The catastrophe date for this pond is when it freezes up completely so the water limitation caused by complete pond drying is not normally experienced by this population. The timing of onset for this population in 1995 and 1996 has been on average, six weeks after the point at which the daylength begins decreasing. As in Hill pond, this environment persists long after the switch to diapausing egg production leading me to believe that the cue for the induction of diapausing egg production in this population might be provided by some local pond condition that varies among years.

Lac Geai is the only clearly permanent habitat and the population at this site hatch from eggs in mid-summer, overwinter under the ice and mate in late spring after the ice clears. Adults of this population were absent from all samples collected in early summer and remained absent for several weeks until the generation resulting from the spring mating finally matured. Some individuals collected during the 1995 sampling did produce some diapausing eggs but the overall population did not ever switch to the production of diapausing eggs over the three year period of data collection.

Overall, it would appear that onset of diapause egg production in the ponds of intermediate permanence (Hill and Stoneycroft) is initiated by local conditions rather than photoperiod, or that selection to react differently to photoperiod has occurred in each pond.

### Pond Physical Conditions

The differences in Stoneycroft that were found between years in the timing of diapause onset in 1997 could not be related to any differences in the physical condition data. The levels of both mean conductivity and mean total dissolved solids were lower in 1996. There were no significant differences found between years in temperature.

### Timing of Diapause Onset

The timing of diapause onset differed between the experimental populations and the natural pond populations for the temporary environments Quarry, Hill and Stoneycroft. The populations from the permanent Lac Geai environment produced very few diapausing clutches over the duration of the sampling season and over the duration of the induction experiment. This would seem to imply for the temporary populations at least, that the removal of these animals from their home environment and holding them under controlled laboratory conditions prevents them from detecting the same cues that the populations in the home ponds are utilizing to time the switch to diapausing egg production.

The different timing of diapause onset exhibited by the populations in the laboratory experiment gives support to the hypothesis that there are specific cues provided by the local environment that are responsible for the induction of diapause other than temperature and photoperiod. This aspect of my data supports the experimental results of Hairston and Olds (1987) in which laboratory-reared animals were induced to begin production of diapausing eggs at a different date than the natural populations which provided the eggs for rearing.

## Photoperiod

The timing effect of photoperiod in other species varies depending upon the species in question and the type of environment that it inhabits. For a freshwater cyclopoid copepod, Watson and Smallman (1971) found short daylengths to induce diapause at many temperatures. The marine calanoid *Labidocera aestiva*, (Marcus 1980, 1982a,b) is induced to begin producing diapausing eggs by short photoperiods under laboratory conditions.

For freshwater calanoid copepods, Hairston & Olds (1984) found that *Diaptomus sanguineus* individuals reared under summer conditions (LD warm) produced more diapausing eggs than winter conditions (SD cool). Walton (1985) reared populations of *Onchodiaptomus birgei* and determined that females at a given density produced about the same proportion of diapausing eggs regardless of the photoperiod.

The *D. leptopus* populations in the laboratory experiments of this study showed significant differences in the production of diapausing eggs within each of the pond populations between treatments. The short-day cold treatment resulted in the greatest output of diapausing eggs for all four ponds but this particular condition is only experienced in nature by the overwintering populations of Lac Geai. The permanent pond populations from Lac Geai always produced fewer diapausing eggs than the other ponds under all conditions.

My results lend some support to the hypothesis that short-day photoperiods can affect the induction of the production of diapausing eggs as reported by Watson and Smallman (1971) and Marcus (1980, 1982 a,b).

## Temperature

For the populations from Stoneycroft and Hill pond, cold temperature appears to be a modifying factor in delaying the induction of diapause since the output of diapausing eggs is significantly lower in the long-day 10°C treatment than any of the other three treatments. Under natural conditions, this would make perfect ecological sense since a pond that was still cool when the daylength is long would have very little chance of drying up and fitness would be maximized by the continued production of subitaneous eggs. The population from Quarry showed no significant differences in egg output in response to temperature and continued to produce mostly diapausing eggs regardless of the temperature and photoperiod combination. The Lac Geai population exhibits an opposing strategy, the animals produce mostly subitaneous eggs regardless of the experimental conditions.

Watson (1986) defines four potential types of diapause/development responses to different photoperiod and temperature combinations; daylengths shorter than a particular value either induce diapause (type I & II) or allow continuous development (type III & IV) while low temperatures can either enhance diapause (type I & III) or enhance continuous development (type II & IV). He states that by combining alternative responses to both temperature and photoperiod, the copepods are able to maintain a high degree of flexibility in the induction of diapause and this is probably the mechanism responsible for the variable responses occurring in populations from different ponds in the locality that he studied.

## Role of the Environment

It is interesting to note that in the Quarry population, a small proportion of animals always continued to produce subitaneous eggs well after the induction date on up to the total

evaporation of the pond. Although the onset of diapause is considered to be when only 50% of the population has switched over to the production of diapausing eggs and there is a possibility that 50% of the population could still be producing subitaneous eggs, the tendency is for populations to switch quickly and completely to 100% diapause at onset as seen in the population of Hill pond (Fig. 2a) and other freshwater temporary ponds (Carter 1974, Hairston & Olds 1984).

This tendency implies that there is a portion of the Quarry population that are engaging in bet-hedging as far as maximization of reproductive potential. By producing hatching eggs after the majority of the population has switched to diapausing eggs, this phenotype would have a maximum gain in years that the pond dries late enough to allow the newly-hatched cohort to develop to adulthood and produce resting eggs before the catastrophe date. Although these individuals would waste their late clutches in years that the pond dries early, this phenotype continues to persist in the population.

#### Other factors

Food availability has been shown to influence the production of diapausing eggs by the calanoid copepod *Onchydaptomus birgei* in the lab (Walton 1985). High levels of food availability resulted in significantly higher production of diapausing eggs than populations reared with low levels of food. The animals in the lab portion of this study were maintained with high level of food availability at all times with all treatments receiving identical quantities at each feeding. Since I did not manipulate food levels in my experiment, I have no results to compare.

Calanoid copepods have been found to produce diapausing eggs after first producing one or more clutches of subitaneous eggs (Watras 1980, Walton 1985) as a function of age. I have found no evidence in my study populations that more subitaneous eggs are produced in the first two clutches in comparison to the later clutches.

### Minipause

Some of the eggs produced by the mating pairs from Lac Geai and thought to be diapausing instead hatched within 7 - 11 weeks after collection when stored at ambient laboratory temperatures. This type of slow-hatching egg has been observed in the marine calanoid copepod *Pontella mediterranea* in which the eggs under study had membrane structures similar to those of diapausing eggs but did not need to undergo a long refractory period before hatching could occur (Santella and Ianora, 1990). The rotifer *Synchaeta pectinata* produces an amictic egg with membrane structures similar to that of a mictic egg but will enter arrest for only a short time (Gilbert and Schreiber, 1995). In cladocera, Doma (1979) found that the ephippial eggs produced by a culture of *Daphnia magna* did not seem to have a resting phase before hatching while Stross (1996) found similar results with a *D. pulex* culture obtained from a biological supply house.

The behaviour of the slow-hatching eggs from the population of Lac Geai found in this study adds to the growing body of evidence that there are differing degrees of diapause in zooplankton.

## **Conclusions**

The degree of permanence of the environment is likely an important factor in the onset of diapause egg production for the non-permanent environments. The populations of both Quarry and Hill exhibited very consistent timing of diapause onset while the timing of diapause onset in the population of Stoneycroft was found to differ significantly between years. The permanent population of Lac Geai showed very little diapause during the duration of the sampling seasons.

The differences found in the physical and chemical parameters of Stoneycroft could not be related to the differences detected in the patterns of diapausing egg production.

Under controlled conditions, the differences between the non-permanent populations disappeared under some treatments but not in others. Overall, the experimental populations did not follow the same patterns as that of the natural populations.

The results of the pond studies give very little insight into the possible cues involved in triggering the onset of diapausing egg production by the populations in the temporary environments. The results of the diapause induction experiments give indirect evidence that the animals require some exogenous cue or a combination of cues from their home environment in order to induce the onset of diapause egg production and maximize the reproductive potential of the population.

Watson (1986) postulates that the differences in intensity and persistence of diapause found in different-sized waterbodies within the same locality results from a flexible response to gradients of both photoperiod and temperature and my results are consistent with this postulate. Further study is required to determine the proximal factors responsible for the different patterns of diapause egg production found in these three temporary environments.

## References

- Ban, S., 1992. Effects of photoperiod, temperature and population density on induction of diapausing egg production in *Eurytemora affinis* (Copepoda: Calanoida) in Lake Ohnuma, Hokkaido, Japan. *J. Crust. Biol.* 12(3): 361-367.
- Ban, S., and T. Minoda, 1994. Induction of diapause egg production in *Eurytemora affinis* by their own metabolites. *Hydrobiologia* 292/293: 185-189.
- Brewer, R.H., 1964. The phenology of *Diaptomus stagnalis* (Copepoda: Calanoida): The development and hatching of the egg stage. *Physiological Zoology* 27 : 1- 20.
- Carter, J.H.C., 1974. Life cycles of three limnetic copepods in a beaver pond. *J. Fish. Res. Board Can.* 31 : 421-434.
- Danks, H.V., 1987. Insect Dormancy: An Ecological Perspective. Biological Survey of Canada (Terrestrial Arthropods). Ottawa. 439 pp.
- DeFrenza, J., R.J. Kirner, E.J. Maly and H.C. van Leeuwen. 1986. The relationships of sex size ratio and season to mating density in some calanoid copepods. *Limnol. Oceanogr.* 31(3): 491-496.
- De Stasio, B.T., 1989. The seed bank of a freshwater crustacean: copepodology for the plant ecologist. *Ecology* 70(5) : 1377-1389.
- Doma, S., 1979. Ehippia of *Daphnia magna* Straus – a technique for their mass production and quick revival. *Hydrobiologia* 67: 183-188.
- Environment Canada, 1990. Biological test method: acute lethality test using *Daphnia* spp. Environment Canada, Conservation and Prot., Ottawa, ON. Report EPS 1/RM/11, 57p.
- Hairston, N.G. Jr., and E.J. Olds. 1984. Population differences in the timing of diapause: adaptation in a spatially heterogenous environment. *Oecologia* 61 : 42-48.
- Hairston, N.G. Jr., and W.R. Munns, Jr. 1984. The timing of copepod diapause as an evolutionary stable strategy. *Am. Nat.* 123: 733-751.
- Hairston, N.G. Jr., and E.J. Olds. 1986. Partial photoperiodic control of diapause in three populations of the freshwater copepod *Diaptomus sanguineus*. *Biol. Bull.* 171: 135-142
- Hairston, N.G. Jr., and T.A. Dillon. 1990. Fluctuating selection and response in a population of freshwater copepods. *Evolution* 44(7): 1796-1805



- Hairston, N.G. Jr., T.A. Dillon and B.T. De Stasio, Jr. 1990. A field test for the cues of diapause in a freshwater copepod. *Ecology* 71(6): 2218-2223.
- Hairston, N.G. Jr., R.A. Van Brunt. 1994. Diapause dynamics of two diaptomid copepod species in a large lake. *Hydrobiologia* 292/293: 209-218.
- Hairston, N.G. Jr., R.A. Van Brunt, C.M. Kearns and D.R. Engstrom. 1995. Age and survivorship of diapausing eggs in a sediment egg bank. *Ecology* 76(6): 1706-1711.
- Hairston, N.G. Jr., and C.M. Kearns, 1995. The interaction of photoperiod and temperature in diapause timing: a copepod example. *Biol. Bull.* 189: 42-48.
- Hansen, A., 1996. Variable life-history of a cyclopoid copepod: the role of food availability. *Hydrobiologia* 320: 223-227.
- Hutchinson, G.E., 1967. A treatise on limnology. Vol. 2. John Wiley and Sons, New York, New York
- Kleiven, O.T., Petter Larsson and Anders Hobæk. 1992. Sexual reproduction in *Daphnia magna* requires three stimuli. *Oikos* 65 : 197-206.
- Knoechel, R., and C.E. Campbell. 1992. A simple, inexpensive device for obtaining vertically integrated, quantitative samples of pelagic zooplankton. *Limnol. Oceanogr.* 37: 675-680.
- Maier, G., 1992. *Metacyclops minutus* (Claus, 1963) - Population dynamics and life-history characteristics of a rapidly developing copepod. *Int. Revue ges Hydrobiol.* 77 : 455-466.
- Marcus, N.H., 1980. Photoperiodic control of diapause in the marine calanoid copepod *Labidocera aestiva*. *Biol. Bull.* 159 : 311-318.
- Marcus, N.H., 1982a. The reversibility of subitaneous and diapause egg production by individual females of *Labidocera aestiva* (Copepoda: Calanoida). *Biol. Bull.* 162 : 39-44.
- Marcus, N.H., 1982b. Photoperiodic and temperature regulation of diapause in *Labidocera aestiva* (Copepoda: Calanoida). *Biol. Bull.* 162 : 45-52.
- Marcus, N.H., 1984. Variation in the diapause response of *Labidocera aestiva* (Copepoda: Calanoida) from different latitudes and its importance in the evolutionary process. *Biol. Bull.* 166 : 127-139.

- Marcus, N.H., 1990. Calanoid copepod, cladoceran, and rotifer eggs in sea-bottom sediments of northern Californian coastal waters: identification, occurrence and hatching. *Mar. Biol.* 105 : 413-418.
- Nishikawa, T., and E.J. Maly. 1996. Factors influencing the degree of sexual size dimorphism within and among calanoid copepod species. *Oecologia* 107: 490-497.
- Saunders, D.S., 1977. The Biological Clock of Insects. In: *The Insects*. Edited by Thomas Eisner and E.O. Wilson. Scientific American. W.H. Freeman and Co. San Francisco, CA. pp 63 - 70.
- Tauber, M.J., C.A. Tauber and S. Masaki. 1986. *Seasonal adaptations of insects*. Oxford University Press, Oxford, England.
- Taylor, B.E., G.A. Wyngaard and D.H. Mahoney. 1990. Hatching of *Diaptomus stagnalis* eggs from a temporary pond after a prolonged dry period. *Arch. Hydrobiol.* 117 : 271-278.
- Walton, W.E., 1985. Factors regulating the reproductive phenology of *Onychodiaptomus bergei* (Copepoda: Calanoida). *Limnol. Oceanogr.* 30: 167-179.
- Ward, H.B., and G.C. Whipple. 1959. *Freshwater Biology* 2<sup>nd</sup> Edition. John Wiley & Sons Inc. New York, NY. 1248pp.
- Watras, C.J., and J. F. Haney. 1980. Oscillations in the reproductive condition of *Diaptomus leptopus* (Copepoda: Calanoida) and their relation to rates of egg-clutch production. *Oecologia* 45: 94-103.
- Watson, N.H.F., 1986. Variability of diapause in copepods. *Syllogeus Series No. 58*. National Museum of Natural Science. 509-513.
- Watson, N.H.F., and B.N. Smallman. 1971. The role of photoperiod and temperature in the induction and termination of an arrested development in two species of freshwater cyclopoid copepods. *Can. J. Zool.* 49: 855-862.